Tumor Necrosis Factor Blockade: Mechanism of Action

Alice B. Gottlieb[1]

Modulation of the immune response with tumor necrosis factor (TNF) blockers is not a new treatment strategy for many inflammatory disorders; however, relatively little is known about their specific mechanism of action. Understanding the mode of action, pharmacology, and pharmacokinetics of the monoclonal TNF antibodies, infliximab and adalimumab, and the soluble TNF receptor, etanercept, may therefore enable us to account for their differing clinical profiles. The aim of this supplement is to explore the roles and mechanisms of TNF and TNF blockade using *in vitro* pharmacological and *in vivo* animal modeling experiments, and *in vivo* studies of the effects of etanercept on the inflammatory cascade in patients with psoriasis.

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INTRODUCTION

Tumor necrosis factor (TNF) blockers revolutionized the care of patients with multiple immune disorders, including rheumatoid arthritis (Maini *et al.*, 1998; Lorenz *et al.*, 2000; Rau, 2002), psoriasis (Chaudhari *et al.*, 2001; Leonardi *et al.*, 2003; Gottlieb *et al.*, 2004; Patel and Gordon, 2004; Tyring *et al.*, 2006), psoriatic arthritis (Mease *et al.*, 2004, 2005; Antoni *et al.*, 2005), ankylosing spondylitis (Braun *et al.*, 2002), and inflammatory bowel disease (Crohn's disease (Targan *et al.*, 1997; Baldassano *et al.*, 2003; Hanauer *et al.*, 2006) and ulcerative colitis (Akobeng, 2006)). Currently, there are three biologics that block TNF approved in the United States for the treatment of various inflammatory disorders. Infliximab and adalimumab are both monoclonal anti-TNF antibodies; etanercept is a soluble TNF receptor-Fc fusion protein. Despite the clinical availability of these life-altering drugs for many years, relatively little is known about their mechanisms of action in patients. If all TNF blockers were merely ''sponges'', sopping up excess TNF in inflammatory conditions, then one could not explain why the three TNF-blocking biologics show differing efficacy and safety profiles as outlined in Table 1. Understanding the mechanisms of action, pharmacokinetics, and pharmacodynamics may explain the observed differences in safety and efficacy profiles (Table 1).

There are different approaches that can be taken to understand potential mechanism of action differences that may account for observed clinical differences. These include *in vitro* pharmacology experiments, animal modeling and studies in patients treated with TNF-blocking drugs. The effect of TNF blockade on the growth of opportunistic organisms *in vitro*, on granuloma formation, and on the activation of immune defense mechanisms *in vitro* is reported in this supplement.

Wallis *et al.* (published in this supplement) examine the effects of all three TNF-blocking drugs *in vitro* on the immune response to and growth of *Mycobacterium tuberculosis* using human whole-blood cultures. Infliximab and adalimumab reduced the proportion of tuberculosis-responsive cells by 70 and 50%, respectively, and suppressed antigen-induced IFN-γ production by 70 and 64%, respectively. In contrast, etanercept produced no significant effect in these measures. Intracellular survival of *M. tuberculosis* was much higher in whole blood cultured with either monoclonal anti-TNF antibody compared with etanercept. Neither apoptosis nor differential induction of IL-10 was observed in whole-blood cultures with any of the three TNF-blocking drugs. In contrast, Ehlers *et al.* (published in this supplement) could not demonstrate any difference among the three TNF-blocking biologics in the induction of human monocyte death by *M. tuberculosis* infection *in vitro*.

Haider *et al.* (published in this supplement) used gene array analysis to study the effects of etanercept or infliximab when cultured in the presence of human peripheral blood mononuclear cells, which were activated with a combination of anti-CD3 and anti-CD28 antibodies. The expression of only 29 genes was suppressed by both etanercept and infliximab. Etanercept suppressed the expression of 55 unique genes. In contrast, 513 genes were suppressed by only infliximab. The authors stated that differing pharmacologic activity may explain the observed clinical differences between etanercept and infliximab.

[1]*Department of Dermatology, Tufts-New England Medical Center, Boston, Massachusetts, USA*

Correspondence: Dr Alice B. Gottlieb, Department of Dermatology, Tufts-New England Medical Center, 750 Washington St., Boston, Massachusetts 02111, USA. E-mail: agottlieb@tufts-nemc.org

Abbreviations: DC, dendritic cell; LT, lymphotoxin; TNF, tumor necrosis factor

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Table 1. TNF blockade: clinical differences in safety and efficacy[1]

TNF blocker	Efficacy	Safety
Etanercept	In a published clinical trial of Crohn's disease, patients receiving etanercept for 8 weeks did not show a statistically significant response compared with patients receiving placebo (Sandborn *et al.*, 2001; Haraoui, 2005).	Review of the scientific literature suggests that there is a difference in incidence of tuberculosis in patients receiving infliximab versus patients receiving etanercept (Gomez-Reino *et al.*, 2003; Wallis *et al.*, 2004; Akkoc *et al.*, 2006; GEAR Rheumatoid Arthritis Study Group, 2006; Opris *et al.*, 2006; Ryan *et al.*, 2006; Tegzova *et al.*, 2006).
	In published clinical trials, 44–49% of patients receiving etanercept 50 mg twice weekly achieved PASI 75 at week 12 (Leonardi *et al.*, 2003; Papp *et al.*, 2005; Tyring *et al.*, 2006).	
	Statistically significant differences in the PASI 75 response rates between the etanercept 50 mg BIW group and the placebo group were reported as early as week 4 (Papp *et al.*, 2005).	
	In the United States, higher initial dosing is indicated for psoriasis patients compared with psoriatic arthritis or rheumatoid arthritis patients (GEAR Rheumatoid Arthritis Study Group, 2006).	
Infliximab	Effective for Crohn's disease and ulcerative colitis (Targan *et al.*, 1997; Hanauer *et al.*, 2002).	Review of the scientific literature suggests that there is a difference in incidence of tuberculosis in patients receiving infliximab versus patients receiving etanercept (Gomez-Reino *et al.*, 2003; Wallis *et al.*, 2004; Akkoc *et al.*, 2006; GEAR Rheumatoid Arthritis Study Group, 2006; Opris *et al.*, 2006; Ryan *et al.*, 2006; Tegzova *et al.*, 2006).
	In published clinical trials of patients with psoriasis, at week 10, 80–88% of patients receiving the 5 mg/kg dose achieved a PASI 75 (Chaudhari *et al.*, 2001; Reich *et al.*, 2005).	
	More infliximab-treated patients than placebo-treated patients achieved PASI 75 by week 6 (Reich *et al.*, 2005).	
Adalimumab	Has shown clinical benefit in patients with Crohn's disease (Sandborn *et al.*, 2004; Papadakis *et al.*, 2005).	Reports in the scientific literature have not shown that there is a difference in incidence of tuberculosis in patients receiving adalimumab and infliximab (Keystone *et al.*, 2004; Bakleh *et al.*, 2005; Gottlieb *et al.*, 2005; Winthrop *et al.*, 2005; Schiff *et al.*, 2006).

[1]No head-to-head, well-controlled trials have been performed comparing the relative efficacy or safety of infliximab and etanercept.
BIW, twice weekly; PASI, psoriasis area severity index.

Kohno *et al.* (published in this supplement) reported that etanercept formed smaller complexes with TNF than did the monoclonal anti-TNF antibodies. These authors postulated that etanercept was a less flexible molecule than the monoclonal antibodies and therefore steric hindrance prevented formation of larger complexes.

Animal studies can be used to study mechanisms of both efficacy and adverse events. Animal studies of the host response to tuberculosis and other opportunistic infections are reported by a number of investigators in this supplement. TNF is essential for granuloma formation and thus for host defenses against mycobacteria and other granulomatous pathogens (Flynn *et al.*, 1995; Wallis *et al.*, 2004; Ehlers, 2005).

Deepe (published in this supplement) summarizes the dependency of containment of both primary and secondary *Histoplasma capsulatum* infection on TNF. In the presence of murine monoclonal anti-TNF antibodies, there is increased death of mice secondary to *H. capsulatum* infection. The role of TNF and its receptors in the host immune response to *H. capsulatum* was dissected using a number of knockout mouse models.

Studies in patients treated with TNF blockade sequestering agents use blood and target organ tissues that are sampled at multiple time points before and during treatment. An early step in the psoriatic cascade is dendritic cell (DC) activation and maturation, stimulating T-cell activation that leads to a predominant T-helper type 1 inflammatory response. Lymphocytes, DCs, neutrophils, keratinocytes, and possibly other cells produce additional cytokines, growth factors, and chemokines that result in a sustained pattern of inflammation and the formation of psoriatic plaques (reviewed by Gottlieb, 2005; Gottlieb *et al.*, 2005; Lizzul *et al.*, 2005). From gene studies and histological analysis, Tan *et al.* demonstrated in this supplement and elsewhere that TNF/lymphotoxin blockade induced by etanercept interrupts this self-sustaining derangement, reverses cutaneous inflammation, and promotes plaque clearance. This is achieved by the down-regulation of multiple inflammatory pathways. TNF inhibition

also interferes with myeloid CD11c+ DC maturation and emigration, T-cell activation and emigration, and neutrophil emigration (Gottlieb *et al.*, 2005).

These authors also show that uninvolved psoriatic skin contains an increased level of nuclear, activated, phospho-RelA compared with normal volunteer skin, and that within the epidermis of psoriatic plaques, there is overexpression of activated, phospho-RelA as compared to unaffected skin. Nuclear factor-κB, which is intimately associated with IFN-γ, TNF, and the production of IL-12 and 23 among other mediators of inflammation and immunity, appears to play a central function in the psoriatic cascade (Lizzul *et al.*, 2005). Dermal DCs were observed to overexpress activated, phospho-RelA in untreated lesional skin, and to become apoptotic with etanercept treatment. This observation may partially explain the substantially decreased DC numbers associated with response to therapy. Infliximab has been demonstrated to induce apoptosis of T cells in lesional tissue and of circulating monocytes from patients with Crohn's disease (Lugering *et al.*, 2001; ten Hove *et al.*, 2002). Whether apoptosis is induced in rheumatoid synovial cells by TNF blockade is still controversial (Pope, 2002; Smeets *et al.*, 2003). To date, there is no single study that studies the comparative mechanisms of action of all three TNF blockers in a single disease.

Finally, understanding differences in the pharmacokinetics and pharmacodynamics in both blood and target tissues of psoriasis patients treated with TNF blockers is important. For example, it may be that higher concentrations of etanercept are desirable in the skin than in the joints of psoriasis patients because there is more surface area in the skin than in the joints. This may be a subject worth exploring in future studies.

CONFLICT OF INTEREST

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